FABRICATION AND MODIFICATION OF IMPLANTABLE MICRO-PROBES FOR NEURO-STUDIES
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ABSTRACT
In this work, implantable micro-probes for central nervous system (CNS) studies were developed on different types of substrates. The probes which contain a micro-electrode array were designed with several lengths for implantation in different locations of the CNS. The electrode surfaces were modified with nano-scale structures, which could increase the active surface area, in order to enhance the electrochemical performance while maintaining micro-scale dimensions of the sensor probes. The electrodes were made of gold or platinum, and have dimensions of 50×100 μm² or 20×40 μm². The silicon probes were modified by nanowires fabricated with the vapor liquid solid mechanism at high temperatures. With polyimide substrates, the nanostructure modification was carried out by applying concentrated gold or silver colloid solutions onto the micro electrodes at the room temperature. The surfaces of electrodes before and after modification were observed and characterized by scanning electron microscopy and cyclic voltammetry. Various experiments were carried out to investigate the improvement in performance with nanostructures. The electrodes were calibrated with H₂O₂, and electrochemical L-glutamate sensors were realized. Comparisons between electrodes with and without nanostructure modification were conducted showing our methods have enhanced the performance of electrochemical neurotransmitter sensors.

Keywords: Micro-probes, neuro-studies, nanowires, nanoparticles, neurotransmitters.

INTRODUCTION
It’s important to monitor neurotransmitters extracellularly in human CNS in order to understand their roles in various neurological disorders, such as Parkinson’s disease, Alzheimer’s disease, depression, addiction, chronic pain, etc. In the past few decades, several methods have been proposed, investigated and used to detect neurotransmitters in association with neuronal diseases, behavior and responses. While optical approaches were costly, bulky, complicated and not reliable enough, those techniques using microdialysis did not give desired temporal and spatial resolutions. Therefore, electrochemical methods have been widely used recently with different techniques, such as amperometry, chronamperometry, and fast-scan cyclic voltammetry, etc. Utilizing those techniques, it is possible for scientists to obtain in vivo and real-time measurements of neurotransmitters, in order to carry out either short- or long-term studies [1-3].

With advances in micro-fabrication, planar micro-electrode arrays (MEAs) have been used in electrochemical neurotransmitter sensors. The technique has proven to provide advantages of multiple-target sensing and flexibility for functionalized surfaces over the conventional carbon fiber electrodes or metal wire electrodes [4-10]. However, the low output electrical current ranges have presented technical challenges in recording and interpreting the signals. This issue has called for a need to increase the signal levels, in both baseline and sensitivity.

In this paper, we reviewed and reported the development of micro-probes containing thin-film MEAs, which could be used as electrochemical neurotransmitters sensors, based on silicon and polyimide substrates. Several surface modification methods have been proposed and investigated in order to enhance the performance of our devices based on two different substrates. Experiments have been
carried out to demonstrate the capabilities of the probes and compare to show the improvement.

**METHODOLOGY AND IMPLEMENTATION**

The micro-probes were fabricated monolithically and then tailored into individual micro-probes by a laser micro-machining system as described in [2-3]. Printed circuit boards (PCB) were designed to mount the probes with copper wires and silver epoxy. Standard connection pins were soldered to the PCB for easy assembly to the recording system. Figure 1 shows images of the probe tips and the assembled devices.

The output current recorded in electrochemical sensors can be described by [1]

\[ i = nFAC_bM \]

where \( i \) is the electrical current measured, \( n \) is the number of electrons for the specific chemical reaction, \( F \) is the Faraday constant of 96,500 Cmol\(^{-1}\), \( A \) is the active surface area, \( C_b \) is the bulk solution concentration of the analyte, and \( M \) is a term about mass transport of the analyte to the electrode surface. Without physically increasing the 2-dimensional area to maintain the micro scale of the probes, the active surface area can be changed by modification with nanostructures on the surface as they will increase the effective surface in the 3rd dimension, resulting in a gain in the total current output [2].

The nanowire modification to Si probes was based on a plasma-enhanced chemical vapor deposition (PECVD) process using the vapor-solid-liquid (VLS) mechanism [2]. The VLS method was developed by Wagner and Ellis in 1964 and has been proved productive to grow Si nanowires. Our fabrication processes utilizing this technique is illustrated in Fig. 2. The modification to polyimide based probes was done by securely applying concentrated gold (Au) or silver (Ag) colloid solutions onto the micro electrodes with a Hamilton syringe with microscopic guidance. Gold colloid solutions (Ted Pella, Inc.) were concentrated using a micro-centrifuge. Silver nanostructures were created by a chemical reaction between copper film and silver nitrate (AgNO\(_3\)) followed by

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Figure 1. (a) SEM image of the Si probe tip. (b) SEM image of the polyimide probe tip. (c) Assembled devices (left: silicon, right: polyimide).
ultrasonication to obtain the colloidal solution. Figure 3 shows SEM photos of (a) the silicon nanowire-modified surface, (b) the Au-particle-modified surface, and (c) the Ag nanostructures surface.

The surface area of the electrode was characterized quantitatively using cyclic voltammetry (CV). Figure 4 shows the CV curves of the electrodes with and without nanowire modification. The experiment was done in N₂-bubbled 1-M H₂SO₄ solution at a scan rate of 100 mV/s. The surface area increased about 10 times [2].

**EXPERIMENTS AND RESULTS**

The detection of neurotransmitters is based on chemical reactions generating H₂O₂ which is then oxidized to release electrons. The electrons will form an electric current proportional to the concentration of the target neurotransmitter. Therefore, the probes were calibrated with H₂O₂ before modified to become electrochemical neurotransmitter sensors [2-3]. We chose L-glutamate, the most abundant neurotransmitter in the CNS, and dopamine (DA), an important neurotransmitter which has many functions in the CNS, to demonstrate. DA is electroactive, so it can be sensed directly using our electrodes. However, L-glutamate is not an electroactive molecule, it requires the L-glutamate oxidase to initiate the following chemical reaction:

L-glutamate + O₂ + H₂O → 2-oxoglutarate + NH₃ + H₂O₂

L-glutamate oxidase (USbio) was diluted in DI water and then mixed with bovine serum albumin (BSA) and glutaraldehyde to enhance the adhesion to the metal surface. The enzyme mixture was then securely deposited to the working electrode using a 10-μl Hamilton syringe. This step-by-step process was described in details in [2-3]. All the calibrations were done with a 0.05-M PBS solution in a beaker under magnetic stirrer. In experiments with L-glutamate sensors, the temperature of the PBS was controlled at 37°C to have the best performance of the enzyme. Chemicals were added in steps and currents were recorded continuously by a potentiostat. Figure 5 (a) shows a calibration result with H₂O₂ on the silver nanostructure modified probe. Figure 5 (b) shows an improvement in the Au modified electrode compared with the normal one in sensing DA, and Fig. 5 (c) shows the enhancement of the nanowire modified probe in detecting L-glutamate. The performance improvement by the modification of surface roughness was significant.
Figure 5. Calibration results with various concentrations of H$_2$O$_2$ on the Ag nanostructures modified probe compared to the normal electrode surface. (b) Response to DA concentrations on normal and Au nanoparticle modified electrodes. (c) Results of L-glutamate sensors with normal and nanowire-modified surfaces.

CONCLUSIONS

We have developed micro-probes useful for neuroscience studies. The nanostructure modification methods to enhance the performance of electrochemical neurotransmitter sensors were successfully demonstrated. The signal levels and sensitivity were significantly improved.

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References